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The CBS-based W/O emulsion for development of heat-resistant compound chocolate: study on thermal, rheological and physical properties

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Abstract

For developing the heat-resistant compound chocolate, a type of W/O emulsion based on cocoa butter substitute (CBS) was evaluated. The emulsion with a W/O ratio of 45:55 (CBS-E55 sample) was chosen as the optimum emulsion based on heat stability and the smallest range of particle size (0.162-0.258 μm), and the thermal properties close to CBS. The optimum emulsion was then incorporated to compound chocolate formulation at different concentrations including 0 (control), 1(CC1), 2 (CC2), 3 (CC3), and 4 (CC4) (w/w%). The results of the DSC test showed that with the addition of 1 and 2% of the CBS-based emulsion, the melting points of compound chocolates increased. The steady rheological test showed that all compound chocolates had thixotropic behavior and also the control and CC4 samples showed the highest (143.760 Pa) and the lowest (47.692 Pa) yield stress, respectively. In the strain sweep oscillatory rheological test, the CC4 and CC1 samples showed the minimum and maximum linear viscoelastic region (LVR), respectively. The puncture test showed an increment of firmness in CC1, but further addition of water decreased the firmness of samples. The results showed improved sensory and physical properties of compound chocolate at the level of 1 % CBS-based W/O emulsion.

Keywords: CBS-based emulsion, heat-resistant compound chocolate, rheology

Introduction

Real chocolates (also known as couverture chocolate) are made of cocoa butter (at least 31%), emulsifiers, cocoa powder, sugar, and milk powder with fine particles. This product is solid at ambient temperature and is melted at body temperature, 33-34 °C (Prosapio & Norton, 2019). Unlike real chocolate, compound chocolates are made of vegetable fats other than cocoa butter and usually have a less intense cacao flavor and a lower creamy and smooth texture. Chocolates have high antioxidant properties due to having high amounts of polyphenols in cacao. Moreover, chocolate is rich in minerals such as copper, iron, magnesium, and potassium (Cinquanta et al., 2016). Cocoa butter has important roles in creating high-quality chocolate including (1) it gives chocolate a smooth and creamy texture, (2) it helps to stabilize chocolate by preventing it from melting too easily at room temperature while ensuring it melts smoothly in the mouth., (3) it lowers the viscosity of melted chocolate, making it easier to work during the manufacturing process (molding and coating stages), (4) cocoa butter also contributes to the desirable snap and glossy appearance of chocolate. When it is properly tempered, it crystallizes in a way that gives the chocolate a shiny finish and a firm snap when broken, (5) While cocoa butter itself has a mild flavor, it acts as a carrier for the flavors of cocoa solids and other ingredients, enhancing the overall taste experience.

Cocoa butter can crystallize into six distinct polymorphic forms, each with unique properties. These crystalline forms of cocoa butter based on the order of melting point and stability are the following: γ (Form I), α (Form II), β' (Form III), β'' (Form IV or β-IV), β (β-V or Form V) and super β (β-VI or form VI). β-V is the most desirable form for chocolate, providing the best melting point (35-37℃), texture, and snap. Proper tempering of chocolate aims to achieve the β-V crystal structure, which gives chocolate its glossy-shiny surface and firm texture. Super beta (β -VI) is the most stable form, but it takes a long time to form and is not ideal for chocolate production. The polymorphism of cocoa butter plays a crucial role in fat blooming due to the different stability and melting points of its crystalline forms. When chocolate is improperly tempered or exposed to temperature fluctuations, the less stable polymorphs (like β-IV) can melt, the liquid fat migrates through the chocolate matrix and eventually reaches the surface and then recrystallizes into more stable forms (like β-VI) and forms visible white streaks or spots, known as fat bloom. To prevent fat bloom, it's essential to properly temper the chocolate carefully (controlled heating and cooling), use stable fillings, and store the products in a consistent, cool environment (Jahurul et al., 2013).

The chocolate-producing process includes mixing, refining, conching of chocolate paste, tempering (to control crystallization), and molding (Toker et al., 2019). During the tempering stage, the chocolate is carefully heated and cooled to specific temperatures to encourage the formation of stable crystals. Typically, dark chocolate is heated to about 45°C (113°F), cooled to around 27°C (80.6°F), and then gently reheated to 31-32°C (87.8-89.6°F). Cocoa butter is the most expensive ingredient of chocolate and is mostly composed of monounsaturated oleic acid (34%) saturated stearic (34%), and palmitic (27%) fatty acids. So replacing cocoa butter with cocoa butter alternatives (CBAs) is commonly used by manufacturers to reduce the cost of production and also control of physicochemical properties of the final product (Kaur., et al. 2022). Compound chocolate is a product of cocoa, which contains cocoa butter alternatives, instead of cocoa butter. This product doesn't need a tempering process which is a great advantage for it (Raoufi et al., 2012). Cocoa butter alternatives (CBAs) is the common name for fats that could fully or partly replaced by cocoa butter and include three groups: (1) Cocoa butter substitutes (CBSs) are made from lauric fats (like palm kernel oil or coconut oil) and chemically different from cocoa butter but have some physical similarities. They can replace cocoa butter entirely in chocolate products and don't require the tempering process. (2) Cocoa butter replacers (CBRs) are non-lauric fats such as palm oil and hydrogenated vegetable oils (e.g., soybean, cottonseed) and are similar in fatty acid composition to cocoa butter but with different triglyceride structures and hence, have different physical properties. They can replace cocoa butter up to a certain percentage without significantly altering the chocolate's properties and are often used in combination with cocoa butter. (3) Cocoa butter equivalents (CBEs) which are made from non-lauric fats like shea butter or ilipe butter and are very similar in both chemical and physical properties to cocoa butter. They can be mixed with cocoa butter in any proportion without affecting the chocolate's quality and require tempering like cocoa butter (Nike & Kumar, 2014; Biswas et al., 2017).

Crystals of cocoa butter melt at 33-35 ℃, and the stability of chocolate shape decreases above this temperature range which causes a problem in areas with hot climates. Heat-resistant chocolate products are specially formulated to withstand higher temperatures without melting or losing their shape. They can often endure temperatures up to 40°C (104°F) or higher which makes them ideal for hot climates and for use in products that might be exposed to higher temperatures during storage or transport (Suri & Basu, 2022).

Production of heat-resistant compound chocolate is one way to overcome the heat-instability problem. There are three main methods to produce heat-resistant compound chocolate, 1. Improvement of the microstructure of materials by adding polyols like sorbitol or glycerine and some sugar like trehalose and palatinose which can bind water, (2). Compatible biopolymer addition such as ethyl cellulose to make a strong network with the lipid phase and (3) Increasing the melting point of the lipid phase using modified cocoa butter or other vegetable fats that have higher melting points (Storz and Marangoni, 2011). The addition of a cocoa butter substitute instead of cocoa butter is one way to increase the melting point. Also, water addition increases the melting point, but it couldn't be added directly into compound chocolate. Because it causes granulation and lumping, sugar blooms, which affects the rheological properties of the final product negatively, resulting in a rough structure in the chocolate compound, and also it could increase microbial activity in the final product (Beckett et al., 2010). Emulsifying the available water in compound chocolate as a W/O emulsion is the solution to this problem which helps to obtain suitable flow properties in compound chocolate (Sullo et al., 2014).

So, the current study aimed to the development of a type of heat-resistant compound chocolate through the incorporation of the emulsions of water in CBS (instead of using cocoa butter). The modification of the water phase in this emulsion was carried out using whey protein concentrate (WPC) and fructose syrup to prepare a more stable W/O emulsion. This emulsion also had the potential for fortification of the final product with hydrophilic and lipophilic bioactive compounds. The effects of these emulsions were investigated on the thermal, physicochemical, rheological, and organoleptic properties of the resulting compound chocolate.

2. Materials and Methods

2.1. Materials

Low-fat cocoa powder (10-12% fat, 4.5% of moisture content, pH= 8.8± 0.4) (ALTINMARKA, Turkey), sugar (sugar factory of Bistoon, Kermanshah, Iran), soybean lecithin (Cargill, Malaysia), whey powder concentrate (WPC) (HILMAR, USA), cocoa butter substitute (Premium, Malaysia), polyglycerol polyricinoleate (PGPR) (Palsguard, Denmark), vanillin (Panda, China), fructose syrup (HFCS 42) (Golshahd, Isfahan, Iran)

2.2. CBS-based W/O emulsion preparation

CBS-based emulsions were prepared according to Zhang et al. (2011), with some modifications. Briefly, PGPR (9 v/w% oil) and CBS were mixed at 50 ℃ for 30 minutes to form a continuous phase. WPC (15 v/w% water) was stirred with water at room temperature and then fructose syrup was added to the water phase and mixing was continued until the preparation of a smooth solution. To prepare W/O emulsions, the prepared water phase was added dropwise to oil phase in water-oil ratio: 25-75 (CBS-E75), 30-70 (CBS-E70), 35-65 (CBS-E65), 40-60 (CBS-E60), 45-55 (CBS-E55), and 50-50 (CBS-E50) (Table 1). The mixtures were homogenized using a high-speed Ultra-Turrax homogenizer at 15000 rpm, and 50 ℃ for 5 min (Hiedoloph, Schwabach, Germany).

2.3. Preparation of compound chocolates containing CBS-emulsion

Ingredients, including cocoa powder (248 g/kg), sugar (460 g/kg), milk powder (260 g/kg), and some amounts of CBS were poured into a mixture and mixed to achieve a paste with suitable concentration. The paste was moved to the refiner to produce fine particles. Conching was then carried out to achieve a viscose liquid from the paste. Lecithin (2 g/kg), the rest of the CBS (30 g/kg), and vanillin were added in this step. This procedure takes 10-24 hours. Optimum water in oil emulsion was added at 1(CC1), 2 (CC2), 3 (CC3), and 4 (CC4) % (w/w) concentration to compound chocolate in a laboratory mixer. A control sample was prepared without CBS-emulsion addition. Chocolate molding was done at 50 ℃ and then cooled to 7 ℃. Samples were packed into polyethylene packages and kept at 28 ℃ until further experiments (Asghar, et al. 2017). Formulations of prepared compound chocolate samples with different concentrations of optimum CBS-emulsion are presented in Table 2.

2.4. Physicochemical measurements

2.4.1. Colloidal stability assessment of emulsions

Assessment of emulsion stability against gravitational separation was done according to Asghar, et al. (2017). To evaluate the stability of emulsion samples during storage at room temperature, 15 ml of each sample was transferred to falcon. All the samples were analyzed visually for gravitational phase separation during 30 days of storage at room temperature. Freezing and heating stabilities of emulsions were analyzed via freezing (keep in the freezer at -18 ℃ for one day), pasteurization (in Ben Mary at 68 ℃ for 30 min), and sterilization (in an autoclave at 121.1 ℃ and 15 min). Based on stability analyses, emulsion samples were selected to conduct further experiments.

2.4.2. Morphology analysis of W/O emulsion

Optical microscopy (CX23, OLYMPUS, Japan) was used to determine the particle size of selected three formulations. For this aim, samples were melted on a Bain-marie device and were placed on lamellae, and particle size was determined at ×100 and magnification at room temperature (Drelich et al, 2010). With the purpose of particle shape analysis and confirmation of particle size of three selected formulations, a Field Emission Scanning Electron Microscope (MIRA3 FEG-SEM, Tescan, Czech) was utilized. 1 ml of each sample was diluted in a 1:10 ratio with acetic acid. One droplet of each diluted sample was dried on aluminum foil and analyzed via FE-SEM (Tilahun Bekele et al. 2021).

2.5. Differential scanning calorimetry (DSC)

For determination of melting temperature, differential scanning calorimetry (SANAF, Iran) was performed on the selected W/O emulsions (i.e. CBS-E60, CBS-E55, and CBS-E50) and prepared compound chocolate samples containing different concentrations of optimum CBS-emulsion. Briefly, 17 mg of each sample (emulsion and compound chocolates separately) was placed into the aluminum pan, and scanning was done at 20 to 70 ℃ with a temperature rate of 2 min℃/. An empty aluminum pan was considered as a reference sample. (Norton & Fryer, 2012).

2.6. Steady and oscillatory shear rheology

Measurement of rheological properties of CBS-based emulsion-loaded compound chocolate samples and control one, were carried out using a Physica MCR301 Rheometer (Anton Paar, Germany) equipped with a couette flow measuring cell (Ref. DG27/T2000/SS). Rheological properties were analyzed in steady and dynamic oscillatory states. Steady-state analysis was performed at a shear rate of 0.01 to 100 s^{-1} (40℃), to determine shear stress, apparent viscosity, and hysteresis area. Casson model (Eq. (1)) was used to fit the data (Halim et al. 2019).

$$\sigma^{0.5} = \sigma_{0c}^{0.5} + \eta^{0.5}\dot{\gamma}^{0.5} \quad (1)$$

Where σ_{0c}: Casson yield stress (mPa), η_c: Casson plastic viscosity (mPa.s)

To determine dynamic oscillatory rheological properties, strain sweep (at 0.1-100% of strain) and frequency sweep (at a frequency of 0.1-100 Rad/s) tests were conducted.

2.7. Sensory evaluation

. Sensory evaluation of compound chocolate samples was done through a 5-point Hedonic scale where 1 equals extremely dislike and 5 equals extremely like (Subroto et al, 2022). The panelists consisted of 30 members (semi-trained men and women aged 24 to 54) who evaluated the desirability of taste, glossiness, flavor, mouth feel, texture (hardness or softness), appearance, and overall acceptability of different formulations.

2.8. Color evaluation

Color measurement of compound chocolate samples was done to determine the effect of the optimum CBS-based emulsion to compound chocolate ratio on the final heat-resistant product's color properties in comparison to the control sample. This assay was done using a Hunter lab (CHROMA METER CR-400, Japan). Pictures were analyzed using Photoshop 8 software. This software provides L, the amount of darkness and lightness (black=0 and white=100), a, the amount of redness-greenness, and b, the amount of yellowness-blueness (Subroto et al, 2022).

2.9. Water activity

The water activity of the CBS-based emulsion-loaded compound chocolates and the control sample was measured using a water activity meter (Rotonic, Switzerland) at 27 ℃ (Prosapio and Norton, 2019). This device measures the relative humidity of a sample's headspace.

2.10. Moisture content

The moisture content of compound chocolate samples was measured according to (Asghar, et al, 2017). Briefly, 2 g of each sample was weighed and spread on a glass plate. Samples were placed in a 100 ℃ oven (Memmert, Germany) for at least 2 hours and weighed every 30 minutes, during the incubation time. The moisture content of every sample was expressed as g of water per 100 g of chocolate sample and calculated via Eq. (2).

$$\text{Moisture content (\%)} = \frac{\boldsymbol{m_1 - m_2}}{\boldsymbol{m}} \times \mathbf{100} \tag{2}$$

Where m_1 is the initial weight of the sample and plate, m_2 is the weight of the sample and plate after incubation time, and m is the weight of the sample.

2.11. Texture analysis (Puncture test)

A puncture test was performed to evaluate the effect of different portions of the optimum CBS-based W/O emulsion on the texture of compound chocolate samples. Universal Testing Machine (Sanaf, Tehran, Iran) equipped with a 2 mm diameter cylindrical penetration probe (punch), cross-head speed of 60 mm/min, and a maximum penetration depth of 7 mm were used at room temperature. The peak force (the maximum recorded puncture force) was considered as the firmness of compound chocolate samples. Three replicates were used for each measurement (Biswas et al. 2017).

2.12. Shape retention stability

Shape retention stability of compound chocolate samples was measured based on a quantitative comparison method, described by Asghar, et al. (2017) with some modifications. The width, length, and height of molded samples were measured at four points for every dimension. Samples were placed into a 40 ℃ oven for an hour. The same measurements were taken place, after cooling to room temperature and then compared to measured amounts before heating.

2.13. Statistical analyze

Statistically significant differences between means were calculated by the one-way analysis of variance (ANOVA) and Duncan’s multiple range test at 0.05 signification level by SPSS software.

3. Results and discussion

3.1. Stability of W/O CBS-based emulsions

6 emulsion samples were analyzed for their stability against creaming and thermal treatment. CBS-E75, CBS-E70, and CBS-E65 showed phase separation (sedimentation) after 24 hours of maintaining at room temperature, while CBS-E60, CBS-E55, and CBS-E50 emulsions were physically stable. Then, these samples were kept at -18 ℃ for 24 h and didn't show any variation in their appearance after this period. All emulsion samples showed an intense change in their color, after sterilization while were stable after pasteurization. Based on stability analyses, CBS-E60, CBS-E55, and CBS-E50 were selected to perform further experiments.

The stability of w/o emulsion is influenced by several parameters including such as the composition of the oil phase, concentration and type of emulsifier (Chen et al, 2016), homogenization parameters (Li et al, 2013), the ratio of water, and oil phases (Raviadaran et al, 2019). Selection of proper production parameters helps to prepare more stable w/o emulsion with controlled physicochemical properties. Stability of w/o emulsion is a complicated issue in the food matrix because of the presence of ingredients such as NaCl, sugar, amino acids, phenolic acids, vitamins, and other additives in food medium (Zhu et al, 2019). Water content increment reduces the stability of w/o emulsion, which was reported by (Xu et al., 2013) especially due to the viscosity difference between water and oil, and higher mobility of droplets of water. Higher water content leads to higher particle size of w/o emulsion and consequently results in coalescence phenomenon (Septiyanti et al., 2021).

3.2. Shape analysis by optical microscopy

Stable W/O emulsion formulations i.e., CBS-E60, CBS-E55, and CBS-E50 were viewed via an optical microscope to verify the morphology of droplets in emulsions. Results are shown in Fig 1 and confirm the spherical shape of water droplets in the CBS-based emulsions.

Sagiri et al (2014) produced mango butter emulsion gel to replace cocoa butter and confirmed the formation of round-shaped aqueous particles in a lipid matrix with uniform dispersion. They reported larger particle sizes for samples with a higher portion of water. You et al, (2023) developed Arabic gum-stabilized water in cocoa butter emulsion, intending to achieve low-fat chocolate production. Optical observation of prepared emulsions confirmed the formation of round droplets in the emulsion.

3.3. Scanning electron microscopy

Stable emulsion formulations (CBS-E60, CBS-E55, and CBS-E50) were analyzed via scanning electron microscopy (SEM), to the evaluation particle size and morphology more accurately and results are presented in Fig 2. All samples showed a spherical shape and the particle size of water droplets in CBS-E60 varied from 73.20 to 120.38 μm, in CBS-E55 varied from 0.162 to 0.258 μm, and in CBS-E50 varied from 0.63 to 0.89 μm. It's obvious that CBS-E55 had the smallest particle size. Small particle size is an important indicator of emulsions' physical stability according to Stokes' law. Higher mobility of water droplets in W/O emulsion will result in higher instability due to an increase of creaming, sedimentation, flocculation, and coalescence phenomena, so the proper ratio of water/lipid phase, lipid phase type, sufficient use of emulsifier and preparation parameters will prevent instability (Zhu et al, 2019). In the current study, the physical stability of the selected emulsion samples could be related to a higher portion of whey protein concentrate in the water phase, which increased the viscosity and consequently reduced the mobility of water which in turn helped to prevent the phase separation. A proper amount of emulsifier (PGPR) also reduces the interfacial tension between water and oil phases, and it results in higher stability of emulsion (Ushikubo & Cunha., 2014). On the other hand, proteins could also act as surface-active components and good emulsifying properties of whey protein have been reported previously (Zhang et al., 2021). Norton & Fryer (2012) confirmed the spherical shape of water droplets in cocoa butter-based W/O emulsion samples. Prosapio & Norton prepared water in cocoa butter emulsion to prepare low-fat chocolate. Cryo-SEM images of emulsions showed spherical shells of fat around water. A study of water in cocoa butter emulsions was taken place via cryo-SEM by di Bari et al, (2019). They confirmed the formation of water droplets surrounded by a crystalline layer of cocoa butter. They suggested that emulsions interact with bulk networks via weak van der Waals forces.

3.4. Determination of melting point of CBS-based emulsions

The water addition can increase the melting point of chocolate but it couldn't be added directly into compound chocolate. Because it causes granulation and lumping, sugar blooms, and also it could increase microbial activity in the final product (Beckett et al., 2010). Emulsifying the available water in compound chocolate is the solution to this problem. Differential scanning calorimetry of stable W/O emulsion samples (CBS-E60, CBS-E55, and CBS-E50) has been shown in Fig 3(a). The melting point of CBS-based emulsion samples increased from CBS-E60 to CBS-E50 (33.8 to 36.4 ℃.) which had a similar trend with increasing water content in these samples. There was a significant difference between CBS-E60 and the other two samples, but there wasn't a significant difference between CBS-E55 and CBS-E50. Contrary to current research, Norton & Fryer (2012), reported no significant difference between the melting point of different formulations of water in cocoa butter emulsion. Wan Aidah et al., (2014), reported higher onset melting temperature for cocoa butter emulsion than pure cocoa butter. Prosapio & Norton (2019), who investigated the melting point of water in cocoa butter emulsions, reported a melting point of 30-34 ℃ for samples with up to 40% water content. An increment of water content up to 50% resulted in a lower melting point in samples which was attributed to the higher thermal conductivity of water in comparison with cocoa butter which led to faster heat transfer.

3.5. Selection of optimum CBS-based W/O emulsion

Based on higher physical stability, spherical shape, small particle size, and a melting point near CBS's melting point, the CBS-E55 emulsion sample was selected as the optimum one and was used to prepare compound chocolate formulations.

3.6. Determination of melting point of compound chocolate samples

Differential scanning calorimetry of formulated compound chocolate samples containing different concentrations of optimum CBS-based emulsion (CBS-E55) and control sample is presented in Fig 3(b). Also, the melting profile of these samples, including T_{onset} (℃), T_{endset}(℃), melting point (℃), and sub-peak area (J/g), is shown in Table 3. T_{onset} indicates the temperature at which crystals start to melt, and T_{endset} shows the temperature at which the liquefaction of samples is completed and the sub-peak area represents the amount of thermal energy that is consumed during melting (Biswas et al., 2017). The samples containing 2% of CBS-E55 (CC2), had the highest T_{onset} (34.6 ℃) and melting point (37.4 ℃). This sample also showed a narrow peak, which shows its suitability to melt in the mouth. The highest $T_{endset,}$ was belonged to CC4 (37.6 ℃), and the highest area represented by the control sample (41.55 mJ). It could be claimed that increasing the CBS-based emulsion content in compound chocolate, improved the melting point of samples until the concentration of 2% to produce heat-resistant compound chocolate. This could be attributed to different mechanisms including (1) the formation of a sugar network; when water is added in the form of a W/O emulsion, it can cause the sugar particles within the chocolate to aggregate and form a network or skeleton. This structure helps to maintain the integrity of the chocolate even when the fat begins to melt (2) formation of capillary forces; the water droplets in the emulsion can create capillary forces that help to hold the sugar particles together (3) decrease of fat mobility; the presence of water can reduce the mobility of the fat within the chocolate. This means that the fat crystals are less likely to move and melt at lower temperatures, thereby increasing the overall melting point of the chocolate. Esmali et al., (2019) reported a higher melting point for chocolate samples fortified with sugar alcohol-contained emulsion than control samples. They declared that the addition of maltitol emulsion (up to %30 of the chocolate) into the samples, increased the T_{onset}. Wan Aidah et al., (2014) declared that the increment of cocoa butter emulsion concentration in chocolate resulted in higher T_{onset}, peak point, and T_{endset} in samples, but the difference wasn't significant. They related this increment to the formation of a strong network inside the chocolate and the entrapment of fat inside it. Aliakbari et al., (2018) utilized gelatin and corn starch to produce heat-resistant milk chocolate. They reported a higher melting point for the chocolate samples containing 10% gelatin and corn starch.

3.7. Rheological properties of compound chocolates

The rheological properties of compound chocolate samples were analyzed to detect the effect of the CBS-based emulsion and its concentration on these samples. For this aim, steady and dynamic oscillatory rheological properties of the control and emulsion-loaded compound chocolate samples were analyzed. In steady state rheological properties, shear stress τ (Pa), and apparent viscosity η (mPa.s) versus shear rate 1-100s^{-1} were measured. In non-Newtonian fluids, viscosity depends on shear rate and it's called apparent viscosity which is shown in Fig 4. Apparent viscosity decreased by increment in concentration of the CBS-based emulsion. The control sample had the highest amount of apparent viscosity (8427 mPa.s), while CC4 had the lowest apparent viscosity (3803 mPa.s) at the shear rate of 1 s^{-1}. In all samples, apparent viscosity decreases by shear rate increment, indicating the shear-thinning behavior of compound chocolate. Kiumarsi et al., (2017) studied the effect of different sweeteners on the rheological properties of chocolate. They showed that apparent viscosity was decreased by an increment of shear rate, so shear-thinning (pseudoplastic behavior) was attributed to all chocolate samples and related to inner structure rupture. On the other hand, the upward and downward curves of shear stress – shear rate in rheograms showed a hysteresis loop, displaying time-dependent thixotropic type rheological behavior (Figure 5). The amount of hysteresis loop area of each sample, which had been calculated via Rheo-compass software, is presented in Figure 6. The hysteresis loop shows the difference in viscosity during the increasing and decreasing shear rates, indicating how the material's structure breaks down and rebuilds over time This shows that the chocolate fluids become less viscous over time when shaken, stirred, or otherwise stressed, and then return to a more viscous state when allowed to rest. The CC3 showed the highest amount of hysteresis area (1504.1 Pa/s.cm^3) and the CC1 sample showed the lowest amount (650.63 Pa/s.cm^3) (Table 7) The amount of hysteresis loop area shows the extent of dependency on time and thixotropic behavior. It depends on particle size distribution, fat content, emulsifier type and concentration, temperature, and shear history (Bursa et al., 2021). Smaller particle sizes increase the surface area, leading to more interactions between particles. This can result in a higher hysteresis area due to greater structural breakdown and recovery. Higher fat content generally reduces viscosity and can decrease the hysteresis area. However, the type of fat and its crystallization behavior can also play a significant role. The presence and type of emulsifiers (like lecithin) can affect the dispersion of particles and the overall rheological behavior, potentially increasing the hysteresis area. Higher temperatures can reduce viscosity and affect the thixotropic behavior, potentially altering the hysteresis area. The pre-shear conditions and the shear rate applied during the rheological measurements can significantly impact the hysteresis area. A higher shear rate can lead to a more pronounced structural breakdown (Ashkezary et al., 2018; Bursa et al., 2021).

Casson's rheological model has been introduced as one of the best models to predict the rheological properties of chocolate (at ＞ 32 ℃) (Kumbar et al., 2021). The Casson model specifically helps in determining the yield stress and plastic viscosity of chocolate, which are important for ensuring the right texture and consistency during manufacturing. In the current research, based on the fitting of rheological data in this model, Casson plastic viscosity and Casson yield stress were achieved for each sample (Table 4). Yield stress in fluid chocolate refers to the minimum stress required to initiate flow. Essentially, it's the point at which chocolate starts to behave like a liquid rather than a solid. This property is crucial for processes like molding and enrobing in chocolate production. The control sample showed the highest Casson yield stress (143.760 Pa) while the CC4 sample had the lowest one (47.692 Pa). With increasing CBS-based emulsion to compound chocolate ratio, the amount of water and PGPR increase in the final product which both have a lubricant effect and cause to decrease in yield stress. The control sample also showed the lowest (0.202 mPa.s) and the CC4 sample had the highest (0.873 mPa.s) amount of Casson plastic viscosity. Farzanmehr et al. (2008) evaluated the effect of sugar replacers on the rheological properties of milk chocolate. They fitted the rheological data in Casson, Bingham, Power-low, and Herschel-Bulkley models, and reported the Casson model as the best model to evaluate chocolate's rheological properties. Biswas et al., (2017) utilized the Casson model to evaluate the CBS-based dark chocolate and reported a decrement in Casson plastic viscosity and an increment of yield stress along with an increment of shear rate. Ashkezary et al., (2018) reported a decrement of apparent viscosity and Casson yield stress value along with particle size increment in compound chocolate samples. Bursa et al., (2021) declared that the size and shape of particles, type of emulsifier, fat amount and distribution, and also process conditions are important factors that affect the flow behavior of chocolate.

In dynamic oscillatory rheological analysis, strain sweep, and frequency sweep tests were conducted. A strain sweep test was performed at the strain of 0.1 -100 % and temperature of 40 ℃to determine the linear viscoelastic region (LVR) (Fig 7(a)). This means that within this region, the stress-strain relationship is proportional and the storage modulus (G') and loss modulus (G") do not change significantly with increasing strain. The storage modulus represents the elastic behavior (solid-like), while the loss modulus represents the viscous (liquid-like) behavior.

The results showed that in the range of 0.01-0.1% of strain, G' and G" curves were linear. The CC4 and CC1 samples showed minimum and maximum linear viscoelastic regions (LVR) in the strain sweep test, respectively. A higher LVR in a strain sweep test indicates that the chocolate's microstructure is more stable and resilient to deformation and break down up to a certain strain level (critical strain). In the LVR, the material's viscoelastic properties remain constant, meaning the internal structure isn't significantly altered by the applied strain. From a microstructural perspective, this stability suggests that the network of fat crystals, cocoa solids, and other components in the chocolate is well-formed and robust. This can be crucial for maintaining the desired texture and mouthfeel of the chocolate. In a strain sweep test, critical strain refers to the strain level at which the material's response transitions from linear to nonlinear behavior. This is crucial for identifying LVR, where the material's properties are independent of the applied strain. Fig 7(b) illustrates the amount of critical strain in the control and emulsion-loaded samples. Results showed that the CC1 sample had the highest amount of critical strain, showing higher mechanical stability.

A frequency sweep test was conducted at a constant strain of 0.01%, angular frequency from 0.01 to 100 rad/s, and temperature of 40 ℃. As frequency increased, both G' and G" increased because the chocolate structure responds more elastically and less viscously to the applied oscillatory stress. This means the material can store more energy (G') and dissipate more energy (G") at higher frequencies. In all samples, the G' values were higher than G" values in all ranges of frequency but were close to each other, displaying weak gel structure formation in the chocolate compounds (Fig 8 (a)). Overall, weak gels demonstrate a more pronounced frequency dependence in their viscoelastic properties (G', G") with a notable balance between their elastic and viscous behaviors.

Fig 8(b and c) comparatively demonstrates G' and G" curves of the control and CBS-emulsion loaded samples at an angular frequency of 0-100 Rad/s. Results showed that the CC3 sample had the highest amount of G' and G" at all frequencies.

The complex viscosity (η*) of all compound chocolate samples tends to decrease with increasing frequency (Fig 8(d). This behavior is typical of materials that exhibit shear-thinning properties, where the viscosity decreases as the shear rate (or frequency) increases. Although G' and G" increase with frequency, the complex viscosity is inversely proportional to frequency and overall resistance to flow (η*) decreases because the chocolate can respond more quickly to the applied stress. The CC3 and CC4 samples showed the highest and the lowest amount of complex viscosity, respectively. Fig 8(e) shows the loss angle which is also called tan (δ) and is the dimensionless ratio of loss modulus to storage modulus ($\tan \delta = G''/G'$). In all compound chocolate samples, loss angles were lower than 1 ($\tan \delta < 1$), indicating that the samples store more energy elastically and behave more like a solid. The loss angle greater than 1 signifies that the material's viscous properties dominate over its elastic properties, making it behave more like a viscous liquid. This means the material dissipates more energy as heat (viscous behavior) than it stores as elastic energy. In all samples loss angle decreased with increasing frequency, displaying the transitions from a more viscous to a more elastic response. At lower frequencies, the molecular chains may have more time to rearrange and dissipate energy, leading to a higher tan δ however, at higher frequencies, the molecules have less time to move, resulting in more elastic behavior and a lower tan δ.

The CC3 and CC4 samples showed the highest and lowest amount of tan δ, respectively, indicating more viscose behavior in CC4 and more elastic behavior in CC3. Kiumarsi et al., (2017), studied the effect of different sweeteners on the rheological properties of chocolate, and the strain sweep test confirmed a more extended linear viscoelastic area for chocolate samples with isomalt. They also showed that the G" values were higher than G' values for a wide range of frequencies (0.1-70 Hz) and suggested weak dispersion for their samples.

3.8. Sensory evaluation

Sensory properties of the control and the formulated compound chocolate samples containing CBS-emulsion were evaluated in terms of appearance, flavor, taste, glossiness, mouth feel, texture, and overall acceptance (Fig 9). Sample CC1 obtained the highest score for appearance, glossiness, mouth feel, texture, and overall acceptance. The control sample showed the highest scores for its flavor and taste. While the CC4 sample had the lowest score for all the tested properties, so it was the organoleptically less desired formulation. Lower scores in the CC4 sample could be due to excessive concentration of CBS-based emulsion in its formulation. Wan Aidah et al. (2014) prepared thermos-resistant milk chocolates and formulated them with cocoa butter emulsion. Replacement of cocoa butter with cocoa butter emulsion reduced the glossiness of chocolates. Also, the overall acceptance of samples decreased with the increment of the cocoa butter emulsion concentration. Asghar et al. (2017) evaluated the sensory properties of CBS-based emulsion-added compound dark chocolates. They reported lower glossiness for the sample with the highest concentration of CBS–based emulsion and related it to sugar crystallization due to higher water content. Biswas et al. (2017) analyzed the sensory properties of the CBS-based dark chocolate. They reported significantly lower acceptancy, especially in terms of taste, for CBS-based dark chocolate compared to cocoa butter dark chocolate at a higher level of CBS.

3.9. Color evaluation

The effect of different ratios of optimum CBS-based emulsions /compound chocolate on color parameters (L, a, b) was evaluated and has been shown in Table 5. It's clear that the highest values for parameter L value, (which refers to brightness/darkness), a value, (which shows the degree of redness/greenness), and b value, (which is an indicator of blueness/yellowness), were presented by the control sample (the sample without added CBS-based emulsion). The addition of CBS-based emulsion into compound chocolate resulted in a decrement of all L, a, and b Hunter parameters, and this decrement increased by increment in concentration of emulsion from CC1 to CC4. Raufi et al, (2012) also showed an overall decrement in color parameters by increasing water and emulsifier concentration, after 30 days of storage at room temperature. In agreement with current research, Mokhtari and Esmaili (2009) reported a higher decrement in lightness in samples, after the incorporation of PGPR. An increment of water content, probably dilutes the medium of compound chocolate and leads to a lower reflection of light and the surface seems darker. Contrary to current research, Asghar et al., (2017) reported no linear relationship between Hunter color parameters and the concentration of CBS-based emulsion in compound dark chocolate.

3.10. Water activity

The water activity of control and formulated compound chocolates containing CBS-based emulsion has been shown in Table 6. The water activity of samples increased by water increment in samples. The control sample had the lowest water activity, while the CC4 sample had the highest amount. The water activity of all samples was lower than 0.6, so they could be considered safe microbiologically. Our result was in the same line with the research work of Prosapio and Norton, (2019) who reported higher water activity in the chocolates containing cocoa butter-based O/W emulsion compared to the control sample. Unlike current results, Asghar et al. (2017) reported the lowest a_w for the sample containing the highest level of CBS-based emulsion and the highest a_w for the control sample. They attributed this phenomenon to the absence of free water in the optimum CBS-based emulsion, and they suggested that PGPR effectively bonds to free water. Esmail et al., (2019) utilized the emulsion containing sugar alcohols to produce heat-resistant chocolate and they reported higher water activity for the formulation with a higher portion of emulsion.

3.11. Moisture content

The moisture content of the control compound chocolate and the samples containing different portions of the optimum CBS-based emulsion are shown in Table 6. The control sample had the lowest moisture content, while the CC4 sample which had the highest amount of water in its formulation, showed the highest amount of moisture content. Asghar et al., (2017) also reported moisture content increment by increasing the level of the CBS-based W/O emulsion. Wan Aidah et al., (2014) reported the highest moisture content for the chocolate sample with the highest portion of cocoa butter-based W/O emulsion. Ashkezary et al., (2018) reported a moisture content of 0.39 to 0.52 for compound chocolate samples prepared with different types of emulsifiers. They declared that moisture content above %1.5 could have a negative effect on the rheological properties of the samples. Esmali et al., (2019) reported higher moisture content for chocolate samples containing sugar alcohol emulsion than the control samples. The highest moisture content was related to samples with the highest portion of sugar and it was due to a higher amount of hydrophilic groups, higher solubility of sugar alcohols in water, and their moisture retention property. Also, the sample containing maltitol and isomalt has a higher moisture content in comparison with sucrose.

3.12. Texture analysis (puncture test)

The texture of compound chocolate samples was analyzed in terms of applied maximum force (N) to make deformation (mm) (maximum penetration depth of 7 mm) and was reported as firmness (also known as hardness or stiffness) (Figure 10). The highest firmness, with 0.86 N was measured in the CC1 sample which means that the addition of a low amount of water into the sample resulted in an increment of firmness. However, the extra amount of water in the CC2 sample, which was due to the increment of the CBS-emulsion ratio in compound chocolate, resulted in the lowest amount of firmness (0.39 N). The CC3 showed nearly the same firmness (0.65 N), and CC4 had lower firmness (0.53 N) than the control sample (0.64 N). Overall it could be concluded that the firmness of compound chocolate decreased with increasing the CBS-emulsion concentration. Raufi et al (2012) declared a reduction in the hardness of compound milk chocolate by the increment of water content at a constant amount of emulsifier and related it to the lubricant effect of water. Wan Aidah et al. (2014) stated that an increment in the concentration of water in cocoa butter emulsion leads to hardness reduction in thermos-resistant chocolates. Biswas et al. (2017), reported a reduction of hardness in chocolate samples with 20% CBS compared to cocoa butter-containing chocolates and related it to the broader melting temperature of it. Asghar et al. (2017) showed hardness decrement in compound dark chocolate by increment in concentration of CBS-based emulsion in it and attributed it to the lubrication property of water and surfactant. Prosapio et al., (2019) utilized water in cocoa butter emulsion to reduce fat in chocolate and reported improved hardness in optimum samples. Ashkezary et al., (2018) investigated the effect of different emulsifiers on the hardness of compound chocolate. They reported hardness of 32.09 to 53.25 N and a decrement of hardness along with an increment in emulsifier concentration.

3.13. Shape retention stability

The shape retention stability of control and compound chocolates containing CBS-based emulsions is determined from the width, length, and height of samples before and after heating. For this aim, three repetitions of each sample were selected and measurements were done at four points (Table 7). A comparison of samples after heating showed that the width of the control, CC3, and CC4 samples decreased but, it increased in CC1 and remained constant in CC2. The length of all samples increased while the height of all samples decreased after heating. CC1 sample showed the most length increment (21 mm), while the most height decrement belonged to the CC2 sample (16 mm). There weren't any significant changes in dimensions after the heating process. Also, there wasn't a significant difference between control samples and ones with different portions of CBS-emulsion. It should be mentioned that the physical appearance of the samples was stable after heating and visual changes were not recognizable. Wan Aidah et al., (2014), compared the shape retention index in normal chocolate and samples prepared with cocoa butter emulsion. They showed that normal chocolate collapsed at 36 ℃ but formulated samples were able to keep their shape up to 40 ℃ .

Conclusion

In the present study, the CBS-based W/O emulsions with different water/oil ratios were successfully prepared with water phase containing WPC and fructose syrup, and oil phase based on CBS- PGPR. The emulsion with a W/O ratio of 45/55 (CBS-E55) was selected as the optimum emulsion and was introduced to the compound chocolate formulation at different concentrations. DSC analysis showed an increment of melting point until the emulsion concentration of 2% W/W. The compound chocolate containing 1% optimum emulsion (CC1 sample) obtained the highest sensory score, and shape stability, and also it had texture firmness close to the control sample. The CC1 sample showed shear-thinning and thixotropic behavior and had the lowest hysteresis area, displaying its ability to structurally recover at the end of applying shear stress. The CC1 sample Also showed the highest amount of storage modulus (G') and the maximum linear viscoelastic region (LVR) which confirms this sample's tendency to keep its shape and structure.

These findings indicate the potential successful usage of the CBS-based emulsion with optimum formulation and concentration as an effective system to produce a heat-resistant compound chocolate with high quality and reduced fat.

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Figure 1. Optical microscopy images of CBS-E60, CBS-E55, and CBS-E50 emulsions.

Figure 2. SEM (scanning electron microscopy) images of CBS-E60, CBS-E55, and CBS-E50 emulsions.

Figure 3(a). Differential scanning calorimetry of CBS-E60, CBS-E55, and CBS-E50 emulsions.

Figure 3(b). Differential scanning calorimetry of compound chocolate samples with different concentrations of optimum CBS-emulsion. (control:sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Figure 4. Apparent viscosity-shear rate rheogram of compound chocolate samples (control: sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Figure 5. Shear stress-shear rate rheogram of compound chocolate samples (control: sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Figure 6. Hysteresis area (Pa/s.cm^3) of compound chocolate samples (control: sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Figure 7. Strain sweep curve (a) and critical strain (b) of compound chocolate samples (control: sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Figure 8. Typical curves of storage modulus (Ǵ) and loss modulus (G) of all samples, comparative curves of storage modulus (Ǵ) (b) and comparative curves of loss modulus (G) (c), complex viscosity (ɳ*) and loss factor (tan δ) at 0-100 angular frequency (Rad/s) in logarithmic scale (control: sample without emulsion, CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Figure 9. Sensory evaluation of compound chocolate samples. (control:sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Figure 10. The maximum force (firmness) in the puncture test of compound chocolate samples (control: sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Table 1. Formulations of the CBS- based W/O emulsions

Ingredient (%) / Emulsion	CBS-E75	CBS-E70	CBS-E65	CBS-E60	CBS-E55	CBS-E50
Water	4.25	8.5	12.75	17	21.25	25.5
Whey powder concentrate	0.75	1.5	2.25	3	3.75	4.5
Fructose	20	20	20	20	20	20
CBS	63.75	59.5	55.25	51	46.75	42.5
PGPR	7.5	8.25	9	9.75	10.5	11.25
Total	100	100	100	100	100	100

Table 2. Formulations of compound chocolate with different concentrations of optimum CBS-emulsion.

Ingredients (%) / Formulations	Control	CC1	CC2	CC3	CC4
CBS	30	29	28	27	26
Sugar	47	47	47	47	47
Cocoa powder	12.4	12.4	12.4	12.4	12.4
Lecithin	0.45	0.45	0.45	0.45	0.45
Milk powder	10	10	10	10	10
PGPR	0.05	0.05	0.05	0.05	0.05
Vanillin	0.1	0.1	0.1	0.1	0.1
CBS-based emulsion	0	1	2	3	4
Total	100	100	100	100	100

Table 3. T_{onset} (℃), T_{endset}(℃), melting point (℃), and subpeak area (J/g) of compound chocolate samples (control: sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Melting profile	Control	CC1	CC2	CC3	CC4
T_{onset} (℃)	25.4	24.2	34.6	20.2	33
T_{endset}(℃)	38.8	40.7	40.2	39	37.6
Melting point (Peak) (℃)	32.1	32.45	37.4	29.6	35.3
SubpeakArea (mJ)	41.55	39.71	36.48	38.67	25.24

Table 4. Casson plastic viscosity (mPa.s) and Casson yield stress (Pa) of compound chocolate samples. (control:sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Sample	Control	CC1	CC2	CC3	CC4
Casson plastic viscosity (mPa.s)	0.202	0.464	0.611	0.443	0.873
Casson yield stress (Pa)	143.760	67.141	97.812	134.908	47.692

Table 5. Hunter color parameters of compound chocolate samples in terms of L, a, b. (control:sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Sample	**L**	**a**	**b**
Control	20	4.5	5.7
CC1	17.4	3.6	3.7
CC2	15.3	3.3	2.2
CC3	15.2	2.6	2.1
CC4	15.3	2.7	2

Table 6. Moisture content (%) and water activity of compound chocolate samples. (control:sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Sample	**Moisture content (%)**	**Water activity**
Control	1.350 ± 0.01^{e}	0.326 ± 0.01^{e}
CC1	1.500 ± 0.02^{d}	0.347 ± 0.01^{d}
CC2	2.250 ± 0.01^{b}	0.363 ± 0.01^{c}
CC3	2.098 ± 0.02^{c}	0.376 ± 0.02^{b}
CC4	2.790 ± 0.02^{a}	0.383 ± 0.01^{a}

Different letters show a significant difference between samples at the 0.05 level of Duncan.

Table 7: Shape retention stability of compound chocolate samples (control: sample without emulsion; CC1:sample with 1% CBS-emulsion; CC2:sample with 2% CBS-emulsion; CC3:sample with 3% CBS-emulsion, and CC4:sample with 4% CBS-emulsion).

Sample / parameter	Width before heat (mm)	Width after heat (mm)	Length before heat (mm)	Length after heat (mm)	Height before heat (mm)	Height after heat (mm)	Weight (g)
Control	22.38 ± 0.14[a]	22.32 ± 0.12[a]	27.23 ± 0.32[a]	27.34 ± 0.08[a]	10.98 ± 0.21[a]	10.86 ± 0.14[a]	6.84 ± 0.21
CC1	22.41 ± 0.11[a]	22.46 ± 0.19[a]	27.30 ± 0.43[a]	27.51 ± 0.31[a]	11.14 ± 0.20[a]	11.05 ± 0.11[a]	7.22 ± 0.29
CC2	22.41 ± 0.12[a]	22.41 ± 0.19[a]	27.31 ± 0.39[a]	27.48 ± 0.38[a]	11.23 ± 0.11[a]	11.07 ± 0.07[a]	7.17 ± 0.28
CC3	22.46 ± 0.08[a]	22.39 ± 0.20[a]	27.33 ± 0.39[a]	27.38 ± 0.40[a]	11.23 ± 0.12[a]	11.10 ± 0.15[a]	7.14 ± 0.26
CC4	22.37 ± 0.13[a]	22.33 ± 0.18[a]	27.26 ± 0.19[ba]	27.37 ± 0.24[a]	11.05 ± 0.21[a]	10.95 ± 0.15[ba]	6.96 ± 0.05

Different letters show a significant difference between samples at 0.05 level of Duncan.

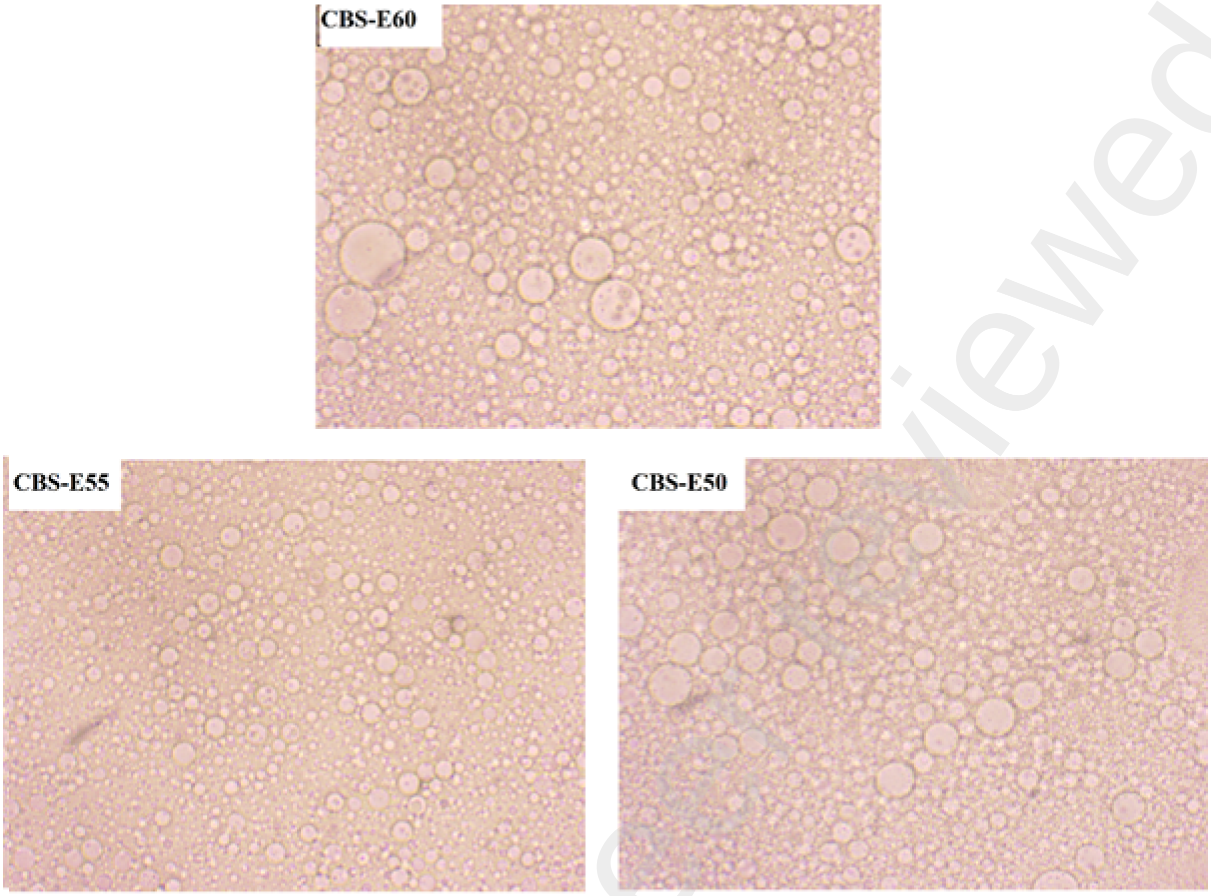

Figure 1.

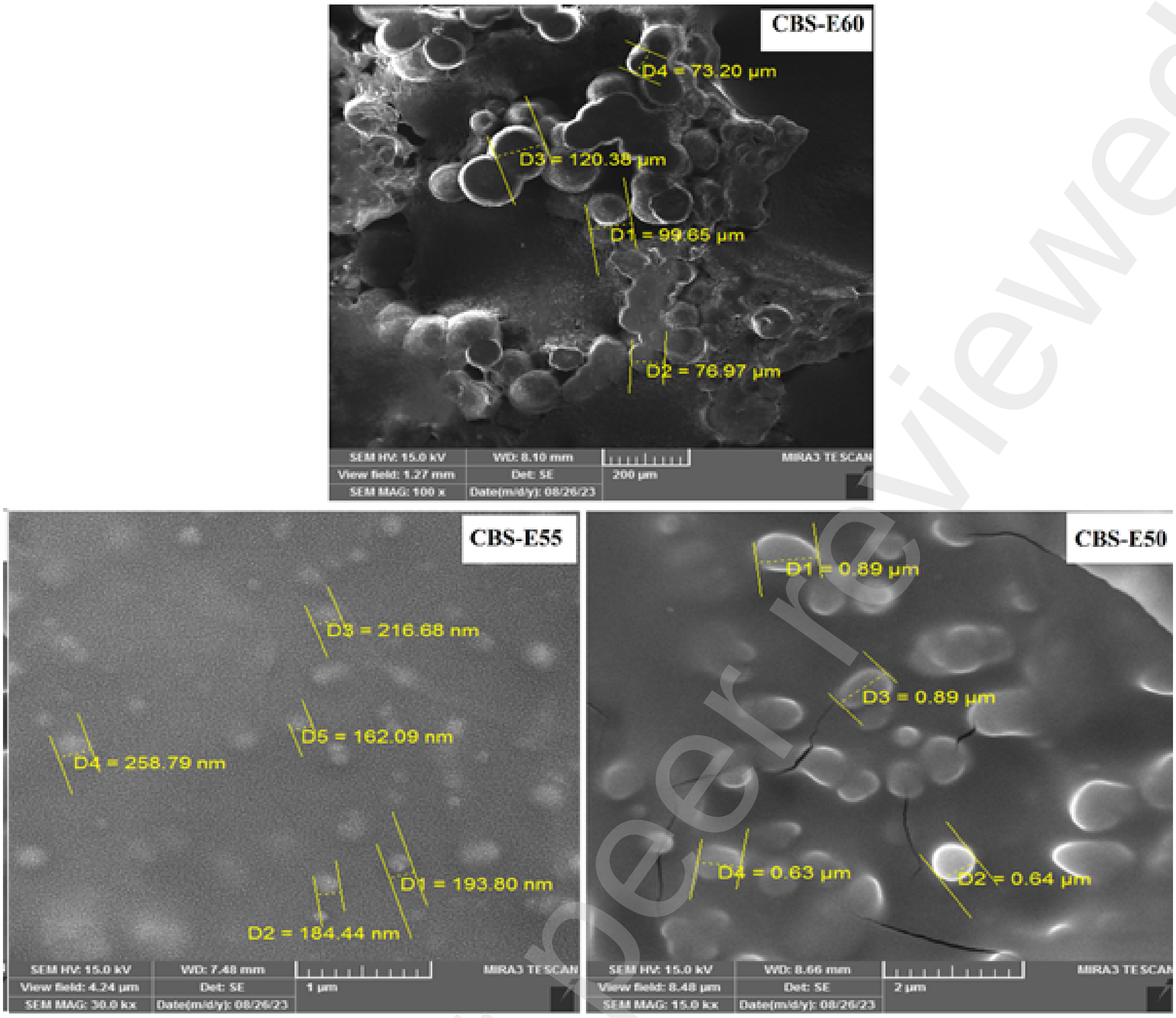
CBS-E60
D4 = 73.20 µm
D3 = 120.38 µm
D1 = 99.65 µm
D2 = 76.97 µm
SEM HV: 15.0 kV
WD: 8.10 mm
MIRA3 TESCAN
View field: 1.27 mm
Det: SE
200 µm
SEM MAG: 100 x
Date(m/d/y): 08/26/23
CBS-E55
D3 = 216.68 nm
D5 = 162.09 nm
D4 = 258.79 nm
D1 = 193.80 nm
D2 = 184.44 nm
SEM HV: 15.0 kV
WD: 7.48 mm
MIRA3 TESCAN
View field: 4.24 µm
Det: SE
1 µm
SEM MAG: 30.0 kx
Date(m/d/y): 08/26/23
CBS-E50
D1 = 0.89 µm
D3 = 0.89 µm
D4 = 0.63 µm
D2 = 0.64 µm
SEM HV: 15.0 kV
WD: 8.66 mm
MIRA3 TESCAN
View field: 8.48 µm
Det: SE
2 µm
SEM MAG: 15.0 kx
Date(m/d/y): 08/26/23

Figure 2.

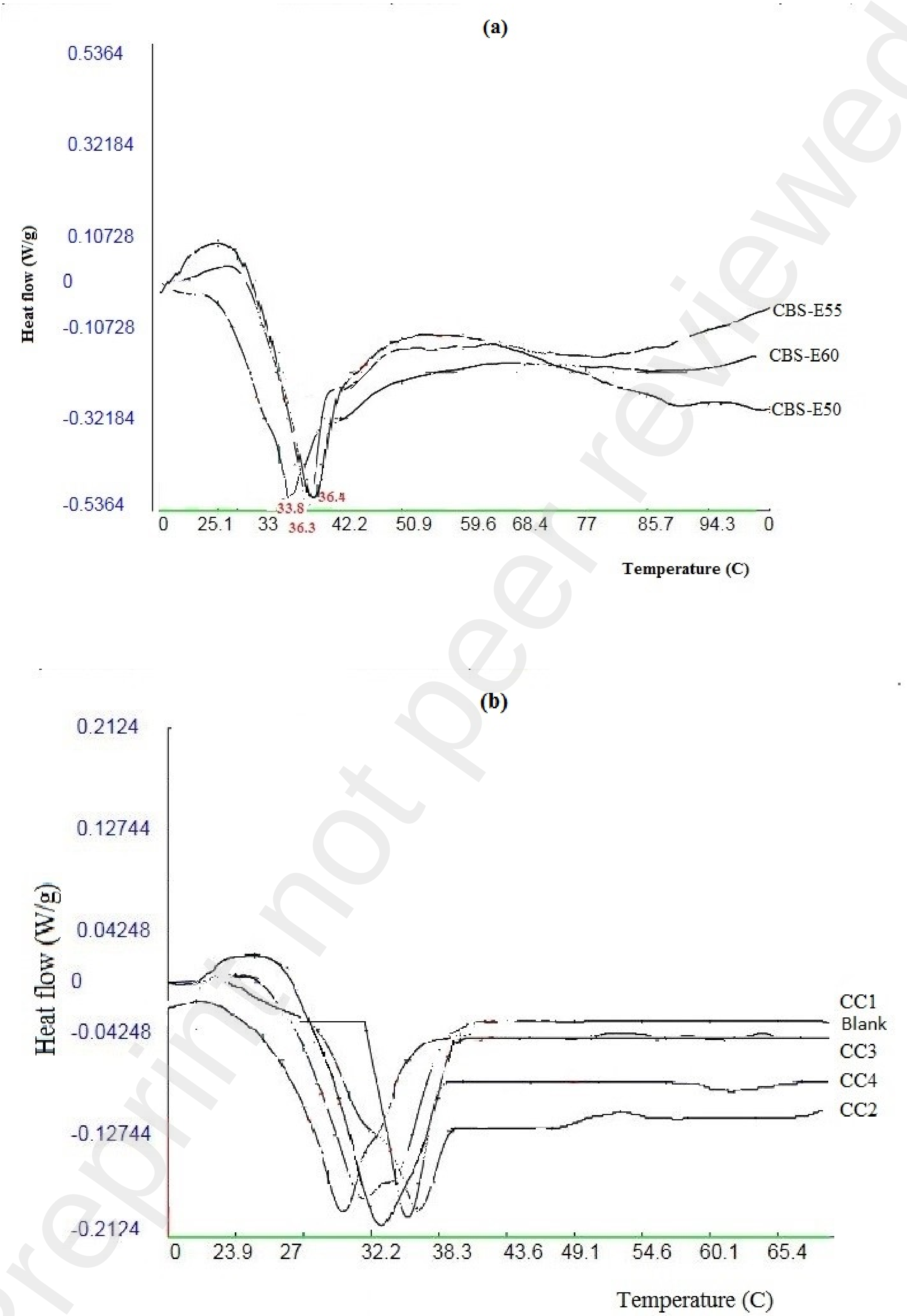
(a)
Heat flow (W/g)
0.5364
0.32184
0.10728
0
-0.10728
-0.32184
-0.5364
0
25.1
33
42.2
50.9
59.6
68.4
77
85.7
94.3
0
33.8
36.3
36.4
Temperature (C)
CBS-E55
CBS-E60
CBS-E50
(b)
Heat flow (W/g)
0.2124
0.12744
0.04248
0
-0.04248
-0.12744
-0.2124
0
23.9
27
32.2
38.3
43.6
49.1
54.6
60.1
65.4
Temperature (C)
CC1
Blank
CC3
CC4
CC2

Figure 3

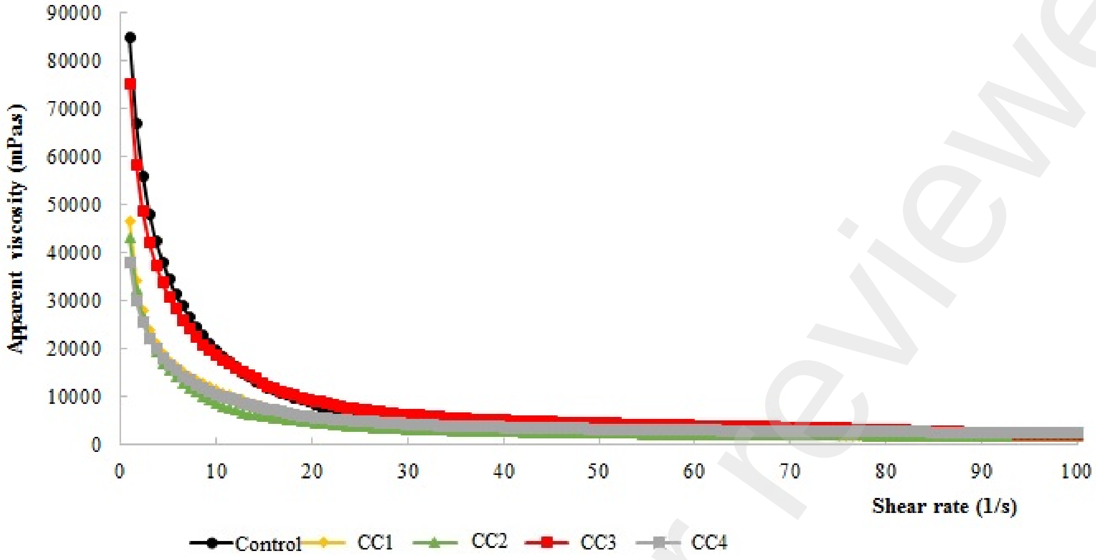
Apparent viscosity (mPa.s)
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
70
80
90
100
Shear rate (1/s)
Control
CC1
CC2
CC3
CC4

Figure 4.

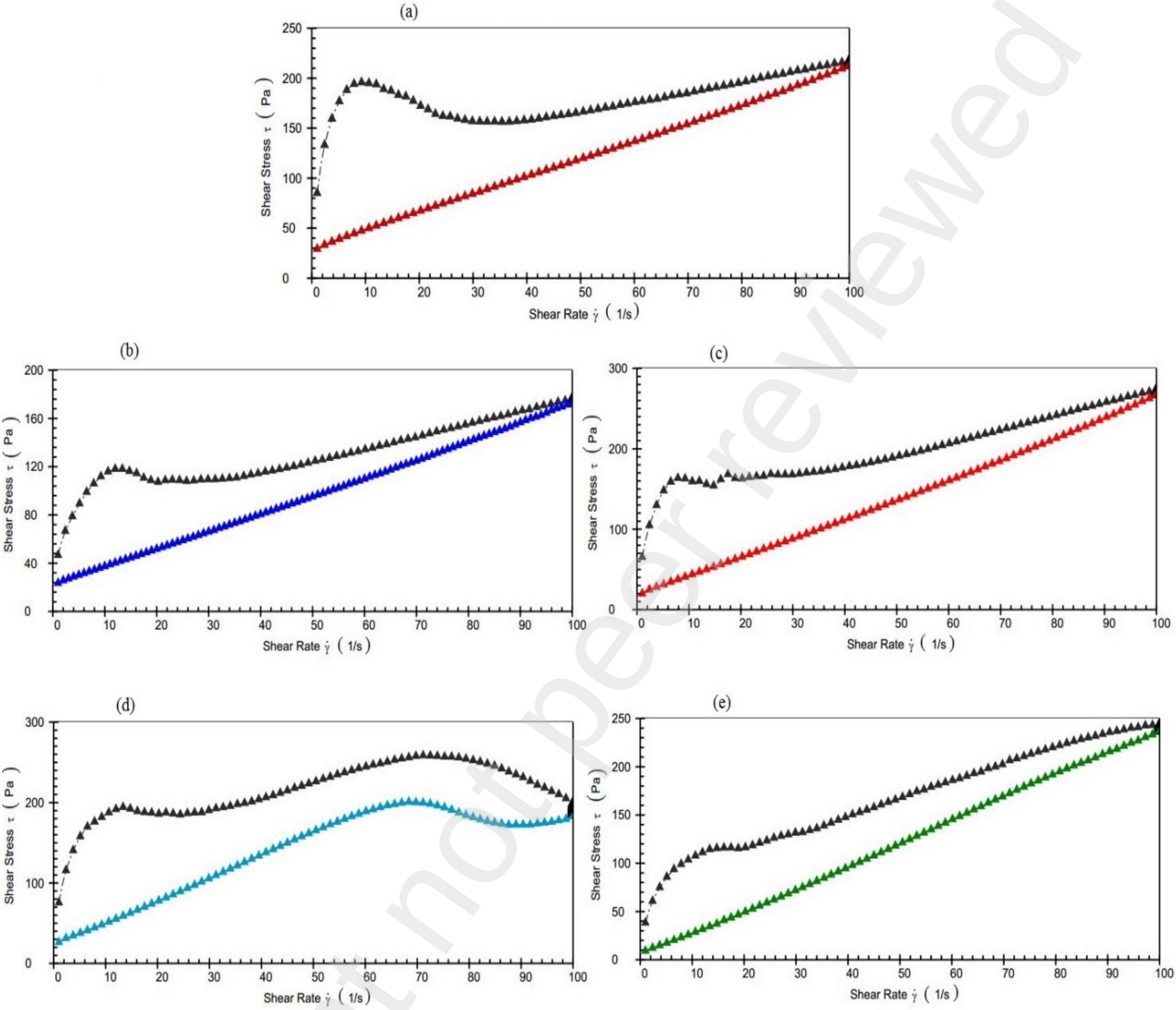
(a)
Shear Stress τ (Pa)
0
50
100
150
200
250
Shear Rate γ̇ (1/s)
0
10
20
30
40
50
60
70
80
90
100
(b)
Shear Stress τ (Pa)
0
40
80
120
160
200
Shear Rate γ̇ (1/s)
0
10
20
30
40
50
60
70
80
90
100
(c)
Shear Stress τ (Pa)
0
100
200
300
Shear Rate γ̇ (1/s)
0
10
20
30
40
50
60
70
80
90
100
(d)
Shear Stress τ (Pa)
0
100
200
300
Shear Rate γ̇ (1/s)
0
10
20
30
40
50
60
70
80
90
100
(e)
Shear Stress τ (Pa)
0
50
100
150
200
250
Shear Rate γ̇ (1/s)
0
10
20
30
40
50
60
70
80
90
100

Figure 5.

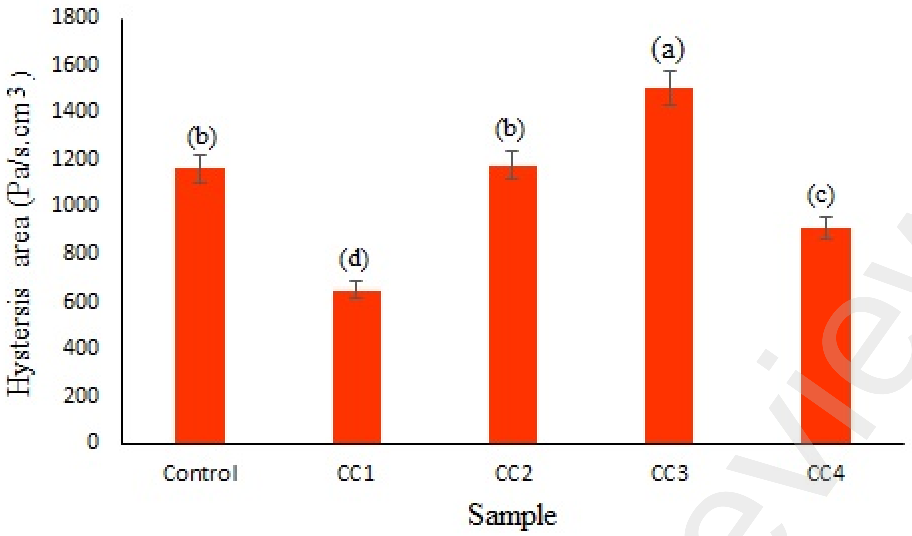
Hystersis area (Pa/s.cm 3)
1800
1600
1400
1200
1000
800
600
400
200
0
(b)
(d)
(b)
(a)
(c)
Control
CC1
CC2
CC3
CC4
Sample

Figure 6.

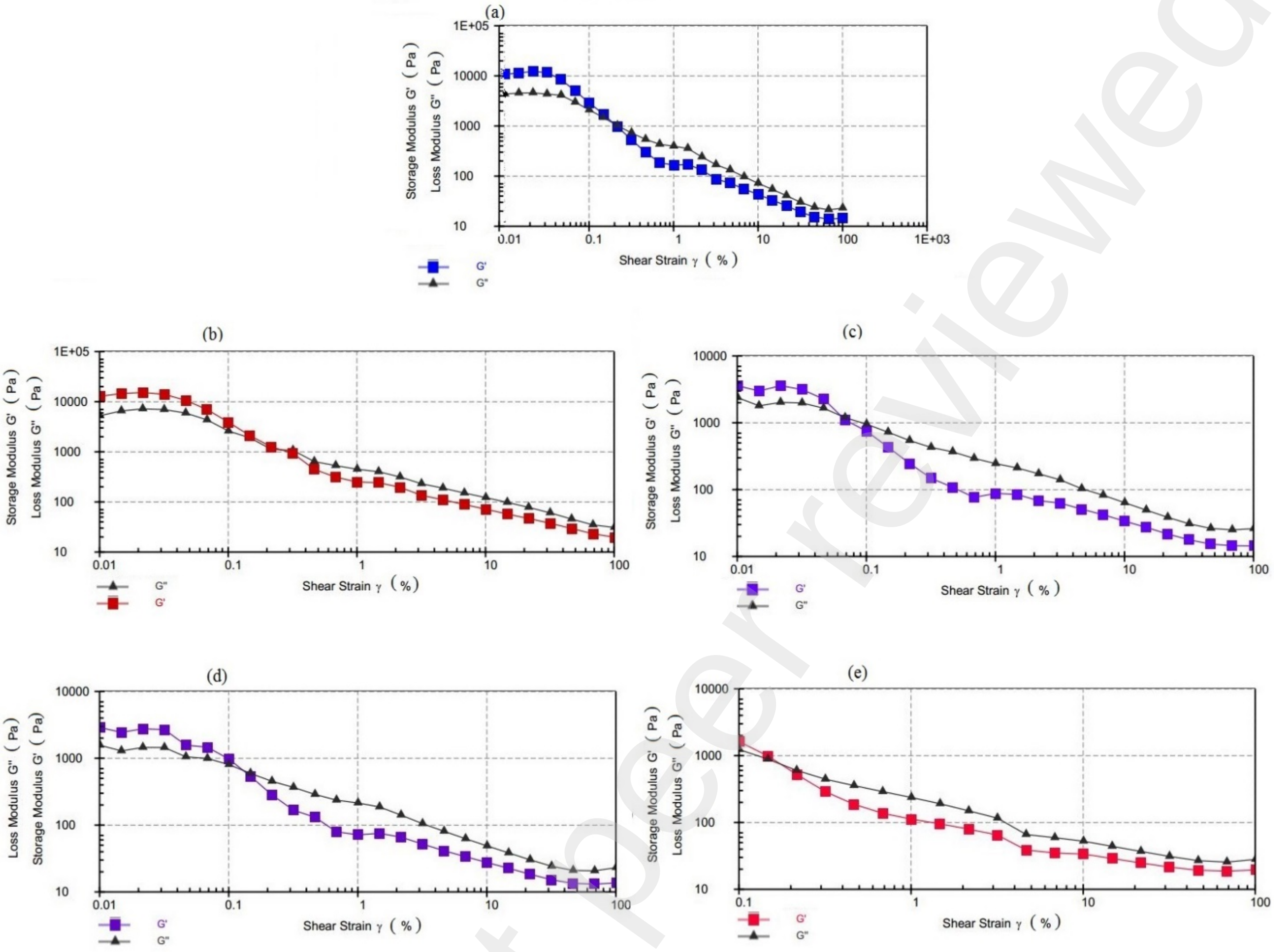
(a)
1E+05
10000
1000
100
10
Storage Modulus G' (Pa)
Loss Modulus G'' (Pa)
0.01
0.1
1
10
100
1E+03
Shear Strain γ (%)
G'
G''
(b)
1E+05
10000
1000
100
10
Storage Modulus G' (Pa)
Loss Modulus G'' (Pa)
0.01
0.1
1
10
100
Shear Strain γ (%)
G''
G'
(c)
10000
1000
100
10
Storage Modulus G' (Pa)
Loss Modulus G'' (Pa)
0.01
0.1
1
10
100
Shear Strain γ (%)
G'
G''
(d)
10000
1000
100
10
Loss Modulus G'' (Pa)
Storage Modulus G' (Pa)
0.01
0.1
1
10
100
Shear Strain γ (%)
G'
G''
(e)
10000
1000
100
10
Storage Modulus G' (Pa)
Loss Modulus G'' (Pa)
0.1
1
10
100
Shear Strain γ (%)
G'
G''

Figure 7 (a).

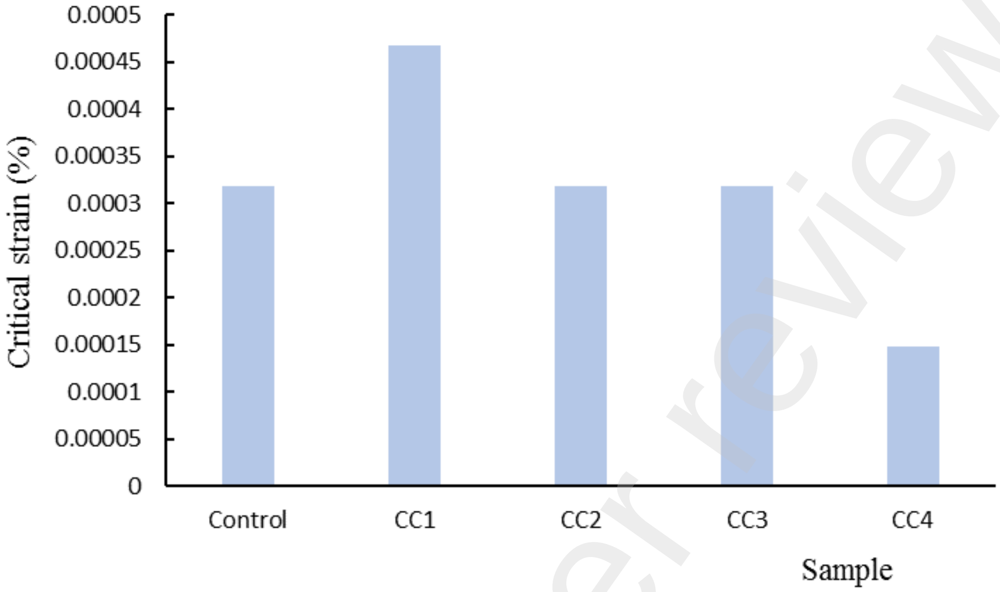
0.0005
0.00045
0.0004
0.00035
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
0
Critical strain (%)
Control
CC1
CC2
CC3
CC4
Sample

Figure 7(b).

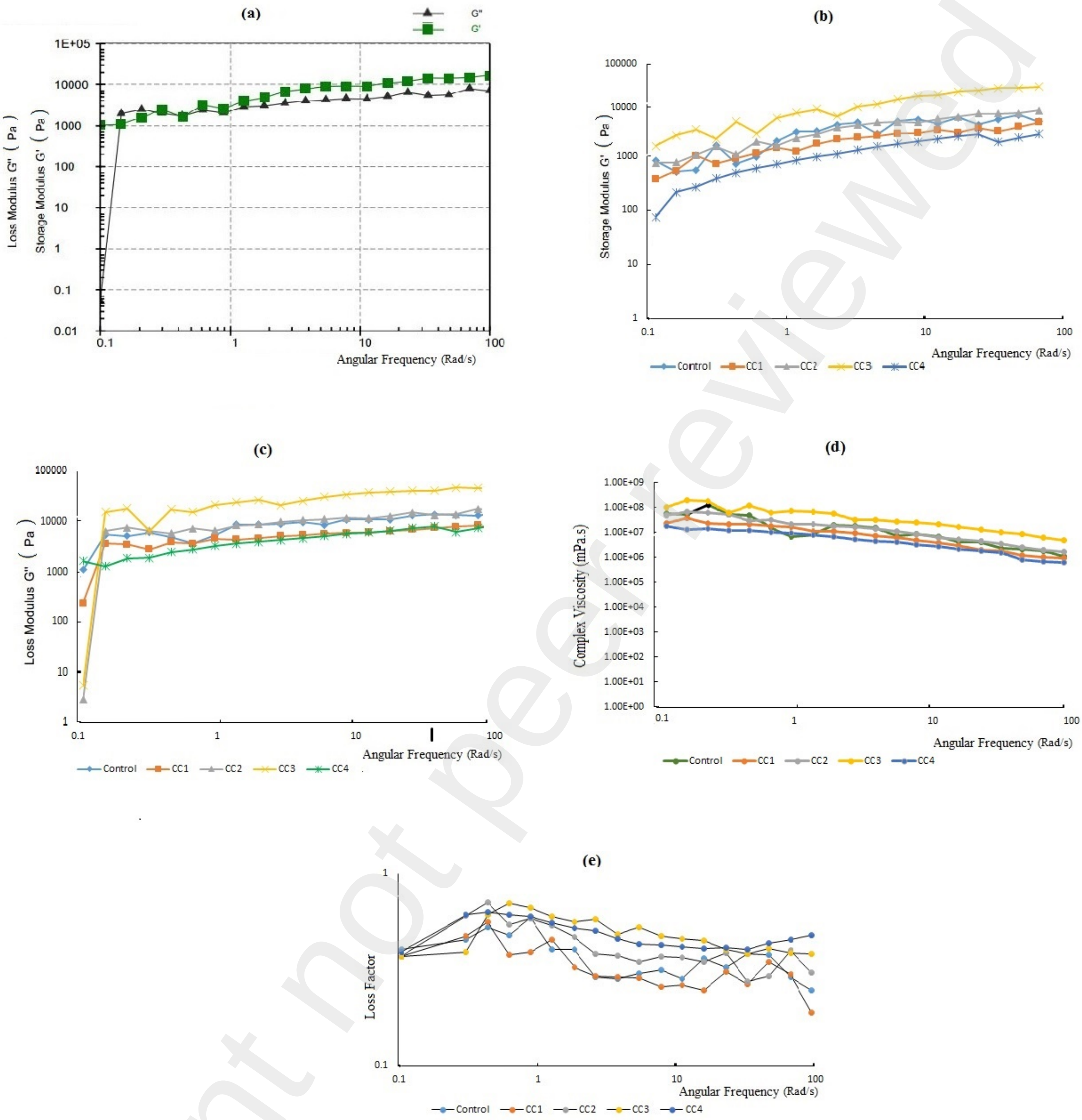
(a)
G"
G'
1E+05
10000
1000
100
10
1
0.1
0.01
Loss Modulus G" (Pa)
Storage Modulus G' (Pa)
0.1
1
10
100
Angular Frequency (Rad/s)
(b)
100000
10000
1000
100
10
1
Storage Modulus G' (Pa)
0.1
1
10
100
Angular Frequency (Rad/s)
Control
CC1
CC2
CC3
CC4
(c)
100000
10000
1000
100
10
1
Loss Modulus G" (Pa)
0.1
1
10
100
Angular Frequency (Rad/s)
Control
CC1
CC2
CC3
CC4
(d)
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Complex Viscosity (mPa.s)
0.1
1
10
100
Angular Frequency (Rad/s)
Control
CC1
CC2
CC3
CC4
(e)
1
0.1
Loss Factor
0.1
1
10
100
Angular Frequency (Rad/s)
Control
CC1
CC2
CC3
CC4

Figure 8.

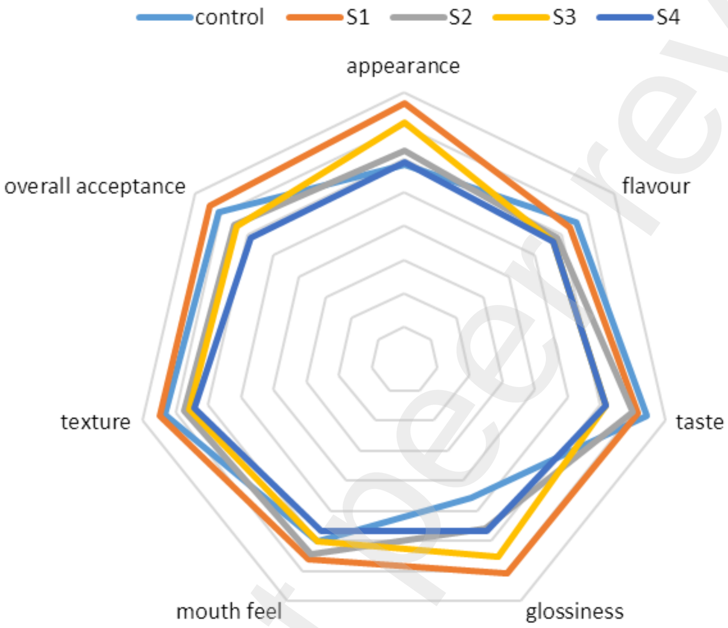
control
S1
S2
S3
S4
appearance
flavour
taste
glossiness
mouth feel
texture
overall acceptance

Figure 9.

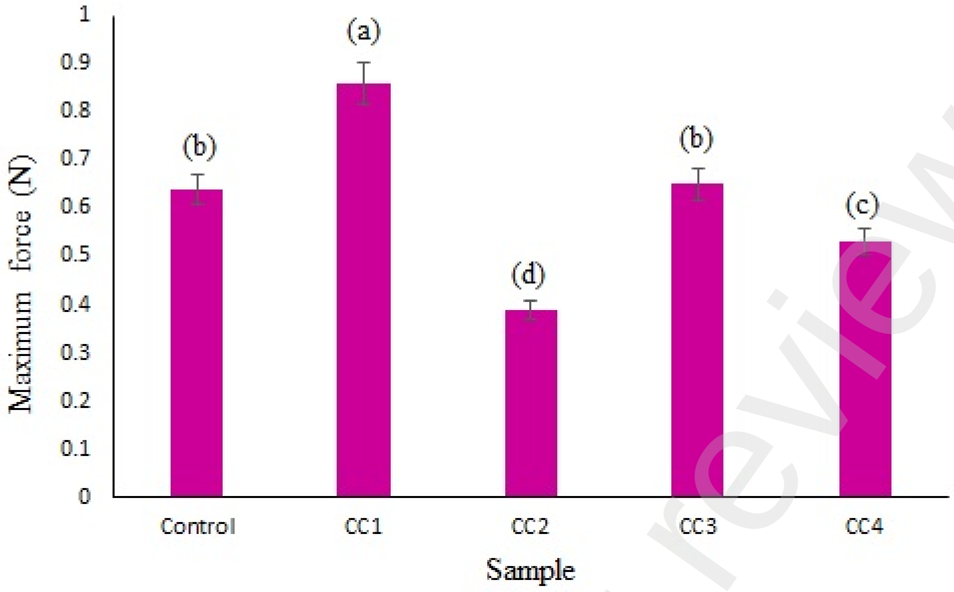
Maximum force (N)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
(b)
(a)
(d)
(b)
(c)
Control
CC1
CC2
CC3
CC4
Sample

Figure 10.